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9 December 2004

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Re: International Application No.: PCT/US2004/027954

International Filing Date: 26 August 2004 (26.08.2004)

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Applicant(s): MINERVA BIOTECHNOLOGIES CORPORATION, ET AL.

Title: TECHNIQUES AND COMPOSITIONS FOR THE DIAGNOSIS AND TREATMENT OF CANCER (MUCI)

Our Reference No.: M1015.70089

CERTIFICATION OF FACSIMILE TRANSMISSION

The undersigned hereby certifies that a Request Under PCT Rule 91.1(b) for Rectification of Obvious Errors with substitute sheet(s) are being facsimile transmitted to European Patent Office, Erhardtstrasse 27, D-80298 Munich Germany, on 9 December 2004.

Donna Lynn Reissig

Name

Signature

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IN THE EUROPEAN PATENT OFFICE
AS INTERNATIONAL SEARCHING AUTHORITY

International Application No.	:	PCT/US2004/027954
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European Patent Office
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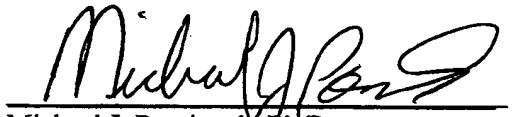
Munich, Germany

REQUEST UNDER PCT RULE 91.1(b)
FOR RECTIFICATION OF OBVIOUS ERRORS

Transmitted herewith for filing is a Request Under PCT Rule 91.1(b) for Rectification of Obvious Errors with substitute sheets.

If the enclosed papers are considered incomplete, the Authorized Officer is respectfully requested to contact the undersigned collect at (617) 646-8000, Boston, Massachusetts.

Respectfully submitted,



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REQUEST UNDER PCT RULE 91.1(b)
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Applicant would like to bring to the Authorized Officer's attention that changes have been made to the above-identified international application.

In the Description of the Drawings

On page 10, line 1, "Fig. 4", as referenced, has been replaced with "Fig. 2";

On page 10, line 5, "Fig. 5", as referenced, has been replaced with "Fig. 3";

On page 10, line 8, "Fig. 9", as referenced, has been replaced with "Fig. 4";

On page 10, line 10, "Fig. 10", as referenced, has been replaced with "Fig. 5", with additional reference therein, on line 11, to "Fig. 9", as referenced, has been replaced with "Fig 4";

On page 10, line 12, "Fig. 21", as referenced, has been replaced with "Fig. 6";

On page 10, line 14, "Fig. 22", as referenced, has been replaced with "Fig. 7";

On page 10, line 16, "Fig. 23" as referenced, has been replaced with "Fig. 8";

On page 10, line 18, "Fig. 24", as referenced, has been replaced with "Fig. 9";

On page 10, line 20, "Fig. 25", as referenced, has been replaced with "Fig. 10";

On page 10, line 22, "Fig. 27", as referenced, has been replaced with "Fig. 11";

On page 10, line 24, "Fig. 28", as referenced, has been replaced with "Fig. 12";

On page 10, line 26, "Fig. 29" as referenced, has been replaced with "Fig. 13";

On page 10, line 28, "Fig. 30", as referenced, has been replaced with "Fig. 14";

On page 10, line 30, "Fig. 31", as referenced, has been replaced with "Fig. 15";

On page 10, line 32, "Fig. 32", as referenced, has been replaced with "Fig. 16";

On page 11, line 1, "Fig. 33", as referenced, has been replaced with "Fig. 17";

On page 11, line 3, "Fig. 34", as referenced, has been replaced with "Fig. 18";

On page 11, line 5, "Fig. 35", as referenced, has been replaced with "Fig. 19";

On page 11, line 7, "Fig. 36", as referenced, has been replaced with "Fig. 20";

On page 11, line 9, "Fig. 37", as referenced, has been replaced with "Fig. 21";
On page 11, line 10, "Fig. 38", as referenced, has been replaced wit "Fig. 22";
On page 11, line 11, "Fig. 39", as referenced, has been replaced with "Fig. 23";
On page 11, line 13, "Fig. 40", as referenced, has been replaced with "Fig. 24",
On page 11, line 15, "Fig. 41", as referenced, has been replaced with "Fig. 25";
On page 11, line 17, "Fig. 42", as referenced, has been replaced with "Fig. 26";
On page 11, line 20, "Fig. 43", as referenced, has been replaced with "Fig. 27";
On page 11, line 22, "Fig. 44", as referenced, has been replaced with "Fig. 28";

In the Detailed Description of the Invention

On page 25, line 29, "31" , as referenced, has been replaced with "15";
On page 27, line 5, "Fig. 21" , as referenced, has been replaced with "Fig. 6", in each instance;
On page 27, line 9, "Fig. 22", as referenced, has been replaced with "Fig. 7";
On page 27, line 13, "Fig. 23", as referenced, has been replaced with "Fig. 8";
On page 27, line 15, "Fig. 24", as referenced, has been replaced with "Fig. 9";
On page 27, line 18, "Fig. 25", as referenced, has been replaced with "Fig. 10";
On page 29, line 29, "Fig. 21", as referenced, has been replaced with "Fig. 6";
On page 29, line 31, "27", as referenced, has been replaced with "11";
On page 30, line 2, "Fig. 28", as referenced, has been replaced with "Fig. 12";
On page 30, line 22, "Fig. 29", as referenced, has been replaced with "Fig. 13";
On page 31, line 24, "Fig. 30", as referenced, has been replaced with "Fig. 14";
On page 31, line 28, "23", as referenced, has been replaced with "8";
On page 31, line 28, "Fig. 21", as referenced, has been replaced with "Fig. 6";
On page 31, line 29, "Fig. 21", as referenced, has been replaced with "Fig. 6";
On page 31, line 30, "Fig. 25", as referenced, has been replaced with "Fig. 10";
On page 44, line 8, "Figs. 31-33", as referenced, has been replaced with "Figs. 15-17";
On page 44, line 16, "Fig. 34", as referenced, has been replaced with "Fig. 18";
On page 44, line 22, "Fig. 35", as referenced, has been replaced with "Fig. 19";
On page 65, line 1, "Fig. 36", as referenced, has been replaced with "Fig. 20";
On page 65, line 4, "Fig. 30", as referenced, has been replaced with "Fig. 14";
On page 65, line 11, "Fig. 30", as referenced, has been replaced with "Fig. 14";
On page 65, line 26, "Fig. 37", as referenced, has been replaced with "Fig. 21";
On page 66, line 1, "Fig. 37", as referenced, has been replaced with "Fig. 21";
On page 66, line 4, "Fig. 37", as referenced, has been replaced with "Fig. 21";
On page 66, line 15, "Fig. 38", as referenced, has been replaced with "Fig. 22";

On page 66, line 22, "Fig. 39", as referenced, has been replaced with "Fig. 23";
On page 67, line 8, "Fig. 40", as referenced, has been replaced with "Fig. 24";
On page 67, line 8, "solid red", as referenced, has been replaced with "grey";
On page 67, line 12, "Fig. 41", as referenced, has been replaced with "Fig. 25";
On page 67, line 13, "Fig. 42", as referenced, has been replaced with "Fig. 26";
On page 68, line 1, "Fig. 37", as referenced, has been replaced with "Fig. 21";
On page 68, line 2, "Fig. 37", as referenced, has been replaced with "Fig. 21";
On page 68, line 6, "Fig. 39", as referenced, has been replaced with "Fig. 23";
On page 68, line 12, "Fig. 39", as referenced, has been replaced with "Fig. 23";
On page 68, line 14, "Fig. 39", as referenced, has been replaced with "Fig. 23";
On page 69, line 18, "Fig. 43", as referenced, has been replaced with "Fig. 27";
On page 69, line 29, "Fig. 44", as referenced, has been replaced with "Fig. 28";
On page 96, line 2, "Fig. 4", as referenced, has been replaced with "Fig. 2";
On page 96, line 4, "Figure 4", as referenced, has been replaced with "Figure 2";
On page 96, line 11, "Figure 5", as referenced, has been replaced with "Figure 3";
On page 96, line 25, "Fig. 9", as referenced, has been replaced with "Fig. 4";
On page 96, line 25, "Fig. 10", as referenced, has been replaced with "Fig. 5";
On page 97, line 4, "Figure 9", as referenced, has been replaced with "Figure 4";
On page 97, line 11, "Fig. 9", as referenced, has been replaced with "Fig. 4";
On page 97, line 17, "Figure 10", as referenced, has been replaced with "Figure 5";
On page 97, line 18, "Fig. 9", as referenced, has been replaced with "Fig. 4";
On page 97, line 21, "Fig. 10", as referenced, has been replaced with "Fig. 5";
On page 97, line 24, "9", as referenced, has been replaced with "4";
On page 99, lines 26 and 27, "Figures 9 and 10", as referenced, has been replaced with "Figures 4 and 5";
On page 104, line 25, "Figure 38", as referenced, has been replaced with "Figure 22";

In Form PCT/RO/101 (supplemental sheet), Sheet No. 4, the filing date provided for U.S. Application Serial No. 09/996,069, listed as filed on "27 November 2002 (27.11.2002)" is corrected to "27 November 2001 (27.11.2001)"

Replacement sheets 10, 11, 25, 27, 29-31, 44, 65-69, 96, 97, 99, 104, and Form PCT/RO/101 (supplemental sheet) are enclosed. Applicant respectfully requests that these replacement sheets be accepted and replace the originally filed pages 10, 11, 25, 27, 29-31, 44, 65-69, 96, 97, 99, 104, and Form PCT/RO/101 (supplemental sheet) to reflect the changes. No new matter has been added.

REMARKS

The Authorized Officer is invited to contact the undersigned by collect telephone call should he/she have any questions concerning this Request.

Respectfully submitted,

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DOCKET NO.: M1015.70089
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Fig. 2 is a graph of percent cell proliferation that shows that an inventive antibody against an epitope of the MUC1 receptor which is proximal to the cell surface, i.e. extracellular, and that dimerizes the receptor, enhances cell proliferation in a manner typical of a growth factor/receptor – antibody interaction;

Fig. 3 is a graph of percent cell proliferation that shows that an inventive antibody against an epitope of the MUC1 receptor which is proximal to the cell surface, and that dimerizes the receptor, dramatically enhances cell proliferation;

Fig. 4 is a silver-stained gel showing ligands that were fished out of cell lysates using a particular PSMGFR peptide, in the presence of the protease inhibitor PMSF;

Fig. 5 is a silver-stained gel showing ligands that were fished out of cell lysates using the PSMGFR peptide of Fig. 4, in the absence of the protease inhibitor PMSF;

Fig. 6 is a graph showing that bivalent anti-PSMGFR antibody stimulates cell growth in MUC1+ breast tumor cell line 1504;

Fig. 7 is a graph showing that bivalent anti-PSMGFR antibody stimulates cell growth in MUC1+ breast tumor cell line 1500;

Fig. 8 is another graph showing that bivalent anti-PSMGFR antibody stimulates cell growth in MUC1+ breast tumor cell line 1500;

Fig. 9 is a graph showing that bivalent anti-PSMGFR antibody stimulates cell growth in MUC1+ breast tumor cell line T47D;

Fig. 10 is a graph showing that bivalent anti-PSMGFR antibody stimulates cell growth in MUC1+ breast tumor cell line BT-474;

Fig. 11 is a graph showing that monovalent anti-PSMGFR inhibits cell growth in MUC1+ breast tumor cell line 1504;

Fig. 12 is a graph showing that monovalent anti-PSMGFR inhibits cell growth in MUC1+ breast tumor cell line 1500;

Fig. 13 is a histogram showing that monovalent anti-PSMGFR competes with bivalent anti-PSMGFR and blocks color change in a nanoparticle assay;

Fig. 14 are western blots showing that breast tumor cells produce MUC1 clevage products of apparent molecular weight 20-30 kDa;

Fig. 15 is a western blot showing that bivalent anti-PSMGFR dimerizes MUC1 in T47D cells and activates intracellular MAP kinase cell proliferation pathway;

Fig. 16 is a western blot showing that bivalent anti-PSMGFR activates intracellular MAP kinase cell proliferation pathway in 1504 breast tumor cells;

Fig. 17 is a western blot showing that bivalent anti-PSMGFR activates intracellular MAP kinase cell proliferation pathway in 1500 breast tumor cells;

Fig. 18 is a western blot showing that drug compounds compete with bivalent anti-PSMGFR and block activation of intracellular MAP kinase cell proliferation pathway;

Fig. 19 is a western blot showing that monovalent anti-PSMGFR competes with bivalent anti-PSMGFR and block activation of intracellular MAP kinase cell proliferation pathway;

Fig. 20 is a western blot showing that breast tumor cells present full-length as well as cleaved MUC1;

Fig. 21 is a western blot showing that MUC1 cleavage products are N-glycosylated;

Fig. 22 is a schematic illustration of the MUC1 receptor variants transfected into HEK cells;

Fig. 23 is a western blot showing a MUC1 tumor-specific cleavage product runs as an approximately 20 kDa band;

Fig. 24 is a histogram showing that monovalent anti-PSMGFR inhibits cell growth in nat-PSMGFRTC transfectants;

Fig. 25 is a western blot showing a bivalent anti-PSMGFR antibody induces ERK2 phosphorylation in HEK cells transfected with nat-PSMGFRTC isoform;

Fig. 26 is a western blot showing a in nat-PSMGFRTC transfectants, bivalent anti-PSMGFR antibody induces ERK2 phosphorylation and monovalent anti-PSMGFR antibody inhibits ERK2 phosphorylation;

Fig. 27 is a western blot showing receptor clevage products for MUC1+ tumor cells and transfectants; and

Fig. 28 is a western blot showing that breast tumor cells may produce two MUC1 clevage products.

Detailed Description of the Invention

Definitions:

The term "MUC1 Growth Factor Receptor" (MGFR) is a functional definition meaning that portion of the MUC1 receptor that interacts with an activating ligand, such as a growth factor or a modifying enzyme such as a cleavage enzyme, to promote cell proliferation. The MGFR region of MUC1 is that extracellular portion that is closest to the cell surface and is defined by most or all of the PSMGFR, as defined below. The MGFR is inclusive of both unmodified peptides and peptides that have undergone enzyme

proliferation, through the well-known MAP (mitogen activated protein) kinase signaling cascade, as indicated by detection of phosphorylation of ERK2 kinase. Significantly, such phosphorylation and proliferation was absent or less evident in similar cells treated with monovalent ligands to MGFR, such as a single chain antibody or a monovalent antigen-binding fragment of antibody.

Cell proliferation may result from accessibility of the MGFR portion to an activating ligand which can interact with the MGFR portion. For example, the self-aggregating IBR of the MUC1 receptor may form a dense reticulum which sterically prevents a ligand such as a growth factor from interacting with the MGFR portion of the receptor, which is proximal to the cell relative to the IBR. In a cancerous or tumor cell, this reticulum may be lost, allowing ligand interaction with the MGFR.

The above mechanistic model is consistent with a mechanism whereby the portion of the MUC1 receptor, that remains attached to the cell surface after shedding of the IBR region or the TPSIBR, i.e. the MGFR, functions as a receptor for ligands that trigger cell proliferation. Evidence is also presented herein that demonstrates that: (a) an interaction between a ligand and a portion of the MUC1 receptor (MGFR), which dimerizes the receptor, triggers cell proliferation; and (b) blocking the interaction of this portion of the MUC1 receptor (MGFR) with its ligand(s), blocks cell proliferation. When tumor cell lines, in which the MUC1 receptor is homogeneously expressed across the entire cell surface, are treated with an inventive IgG antibody raised against the MGFR portion of the MUC1 receptor (e.g. PSMGFR), the rate of cell proliferation is greatly enhanced. Since intact IgG antibodies are bivalent, i.e. one antibody simultaneously binds to two adjacent MGFR portions on the cell surface, these results demonstrate that the antibody acts as an activating ligand, mimicing the effect of a growth factor, which dimerizes MGFR portions, and thus triggers a cell proliferation signaling cascade which is consistent with signaling via the cytoplasmic tails of the receptors. This is further supported by the experiments discussed below showing that dimerization of two adjacent MGFR portions on the cell surface induces ERK-2 phosphorylation indicative of MAP kinase cell proliferation signaling (See e.g. Fig. 15). This finding leads to two conclusions. First, an activating ligand(s) that binds to the MGFR portion of the MUC1 receptor causes inductive multimerization of the receptor. Secondly, an effective therapeutic strategy is therefore to block the MGFR portion of the receptor with a monomeric composition, thus preventing inductive multimerization and subsequent signaling cascades. For example, a single chain, or monovalent, antibody, or a

breast tumor cell lines that express the MUC1 receptor along with control cell lines that did not. The addition of the antibody stimulated cell proliferation only in cells that expressed the MUC1 receptor. The 1504 breast tumor cells that were treated for either 5 or 6 days with the bivalent anti-PSMGFR antibody underwent 400% – 600% enhancement of cell growth, see Fig. 6 and Example 11. Still referring to Fig. 6, control cells K293 and Hela were unaffected by the same dosage of the same antibody, anti-PSMGFR. The shape of the proliferation enhancement curves argue that the antibodies dimerize the receptor; at very high antibody concentrations the rate of cell growth decreases, as each receptor is bound to a single antibody rather than one bivalent antibody bound to two receptors. Fig. 7 shows that cells from the breast tumor cell line 1500 underwent a 200% enhancement of cell proliferation after treatment with the bivalent anti-PSMGFR for 3 days. When bivalent anti-PSMGFR treatment was extended for a fourth day, the percentage enhancement of cell growth increased to 300%, see Fig. 8. Breast tumor cells from the T47D cell line were also tested for the ability of the bivalent anti-PSMGFR antibody to trigger cell proliferation. Fig. 9 shows that these cells also underwent an approximately 125% enhancement of cell growth, and as with 1500 and 1504 cell lines, the response was dependent on the concentration of the antibody. Breast tumor cell line BT-474 displayed similar stimulation of cell growth (150 – 200%) in response to bivalent anti-PSMGFR, see Fig. 10. $MUC1^-$ cell line MDA-MB-453 was not affected by the addition of anti-PSMGFR at any concentration (data not shown).

Monovalent forms of anti-PSMGFR fragments block cell growth in MUC1 positive tumor cells. As previously described, $MUC1^+$ tumor cells are induced to proliferate when the MUC1 receptor is dimerized. Specifically, the signal to proliferate is generated when a portion of the MUC1 receptor proximal to the cell surface is dimerized. As described above, one way in which the receptors can be dimerized is via a bivalent antibody directed against the MUC1 receptor. In a preferred embodiment, the antibody is directed against the MGFR and in yet a more preferred embodiment it is directed against at least a portion of the PSMGFR. As described above and further below, agents that bind to the MGFR portion of the MUC1 receptor in a monomeric rather than dimeric fashion can block the dimerization of the receptor and in so doing inhibit cell proliferation. The discussion below describes several chemical compounds that inhibit the growth of MUC1+ tumor cells by binding to the MGFR portion of the MUC1 receptor.

generate antibodies wherein each recognition fragment of a bivalent antibody binds to different but essentially adjacent sites on the same antigen.

As described in greater detail below, MUC1 monovalent antibodies/fragments described above for use as cancer therapeutics may be polyclonal or monoclonal and may be obtained by immunizing a number of different animal species, i.e. rabbit, goat and the like. Additionally, techniques are known to those skilled in the art for generating hybridoma cells, which then are grown and harvested to yield a supply of antibody without the need for repeated animal immunization. Alternatively, humanized monovalent antibodies/fragments, also described in more detail below, that target these portions of the MUC1 receptor may be used as effective anti-cancer agents that are less likely to invoke immune responses in the patient. Methods of the invention also encompass recombinant methods for antibody and Fab production that do not include animal immunization.

As explained below, methods of generating monovalent antibodies and monovalent antigen-binding fragments of antibodies are known to those skilled in the art. A standard method is the controlled proteolysis of a bivalent antibody. The inventors generated a monvalent PSMGFR-specific antibody fragment by proteolyzing their inventive bivalent anti-PSMGFR. Monovalent anti-PSMGFR competes with the bivalent anti-PSMGFR antibody for the same binding site within the MGFR portion of the MUC1 receptor.

The present invention, in certain embodiments, details how monovalent antibodies/fragments, which are directed against a portion of the MUC1 receptor that is proximal to the cell surface, inhibit the growth of MUC1+ tumor cells. Monovalent antibodies/fragments that targeted the PSMGFR portion of the MUC1 receptor were produced by proteolyzing the bivalent anti-PSMGFR antibody, which has been herein to induce cell growth, presumably by dimerizing the MUC1 receptors. Thus, it follows that the monovalent form of this very antibody would block cell proliferation by binding to the MGFR portion and thus prevent the binding of cognate ligands and/or dimerization.

Herein we provide experimental results that demonstrate that monovalent antibody fragments that target the PSMGFR do in fact inhibit the growth of MUC1 positive tumor cells and have virtually no effect on control cell lines. Referring now to Fig. 6, recall that the addition of bivalent anti-PSMGFR induced a 600% enhancement of cell growth. Fig. 11, the method by which the results were generated being described in Ex. 11, shows that the addition of the monovalent form of the same anti-PSMGFR to a MUC1 positive breast tumor cell line, 1504, had the opposite effect in that cell growth inhibited by about 150%,

which indicates induced cell death. The addition of the monovalent anti-PSMGFR had a similar effect on breast tumor cell line 1500, which is also MUC1$^+$, see Fig. 12.

Monovalent anti-PSMGFR validates the in vitro drug screen; it inhibits the color change of the PSMGFR-immobilized nanoparticles caused by the addition of tumor cell lysates to the nanoparticles, as described in more detail below. It should be noted that the monvalent antibody/fragment also can inhibit the dimerization of the PSMGFR peptide in vitro. Nanoparticle-based drug screening assays to identify compounds that inhibit dimerization of the MGFR portion of the MUC1 receptor are described herein and in commonly-owned U.S. patent application publication no. 2003/0036199; and International Publication No. 02/056022 A2. In certain of these assays, histidine-tagged PSMGFR peptides, (e.g. SEQ ID NO: 2), were immobilized on NTA-Ni++-SAM-coated gold nanoparticles. Lysates and supernatants from MUC1 positive tumor cells, which presumably contain the cognate ligands of the MUC1 receptor, were added to the nanoparticles. Upon addition of the lysate/supernatant mixture, the color of the nanoparticle solution turns from its characteristic pink to blue, presumably when the cognate ligands dimerize MUC1 receptor peptides on two different nanoparticles. The addition of bivalent anti-PSMGFR antibody, in place of the lysate/supernatant solution, also causes the nanoparticle solution to turn from pink to blue, as the bivalent antibody also dimerizes two PSMGFR peptides on different nanoparticles. However, the addition of monovalent anti-PSMGFR to the drug screening assay, to which the lysate/supernatant has also been added, inhibits the color change, presumably by competing with natural, cognate ligands for binding to the PSMGFR peptide. Fig. 13 shows that the characteristic nanoparticle color change that occurs upon the addition of bivalent anti-PSMGFR was inhibited upon addition of the monovalent anti-PSMGFR

The present results also suggest that the MUC1 receptor is involved in apoptosis. The addition of the monvalent anti-PSMGFR not only inhibited cell growth, but also induced cell death. This indicates that the MUC1 receptor also mediates signaling pathways involved in the process of programmed cell death known as apoptosis.

Present cancer research literature presents a confusing picture as to whether or not the overall amount of MUC1 receptor produced by the cell can be correlated to metastatic potential or tumor aggressiveness. The results described herein support the idea that a key mechanism of cell growth in MUC1 positive cancers may depend more on the amount of MUC1 cleavage that occurs rather than the overall amount of MUC1 receptor that is

expressed. Low molecular weight species that migrate on an acrylimide gel with an apparent molecular weight of around 20-30 kD (some glycosylated) exist in MUC1-positive tumor cells but do not exist in sufficient numbers to be detectable in non-tumor MUC1 cells. The inventors identified two cleavage sites of the MUC1 receptor in tumor cells. The first cleavage site occurs in the middle of the IBR and the second cleavage site, which our evidence indicates is the more tumorigenic form, occurs at the C-termial end of the IBR: the first cleavage site being located at the N-terminus of TPSIBR (SEQ ID NO: 8) and the second cleavage site being located at the N-terminus of the nat-PSMGFR having SEQ ID NO: 60. When cleavage occurs at the first site, the portion of the receptor that remains attached to the cell surface is the similar to TSESMGFR (See Table 1, SEQ ID NO. 66, but with the native SRY sequence). When cleaved at the second site, the portion that remaining portion is a PSMGFR as shown in Table 1, SEQ ID NO. 63. This low molecular weight species that is tumor specific consists essentially of the native PSMGFR sequence and in some cases the TSESMGFR sequence and is available to cognate ligands, i.e. not self-aggregated, than on the overall amount of MUC 1 receptor expressed by the cell. Supporting this conclusion, susceptibility of tumor cells to proliferate was found, within the context of the present invention, to be a function of the amount of the shorter form of the MUC1 receptor.

Comparison of the present results generated by western blot analyses, which quantitated the amount of low molecular weight MUC1 species produced by each cell type tested, with the above presented cell proliferation data shows that the susceptibility of the breast tumor cells to antibody-induced cell growth is proportional to the amount of the low molecular weight MUC1 species (25-30 Kd glycosylated; 19-20Kd unglycosylated) that the cell produces. Referring now to Fig. 14, breast tumor cell lines 1500 and 1504 produce a considerably greater amount of the MUC1 cleavage product that runs at 19-20 Kd than the BT-474 BT cells or the control K293 and HeLa cells. Correspondingly, the anti-PSMGFR-induced increase in the proliferation of cell lines 1500 and 1504 was up to about 400% (Fig. 8) and up to about 600% (Fig. 6) respectively, while there was no detectable increase in the rate of cell growth for control cells (Fig. 6) and the growth of BT-474 cells increased by only up to about 200%, see Fig. 10.

In further support of the conclusion that cleavage products of the MUC1 receptor function as growth factor receptors in tumor cells, HEK cells were transfected with MUC1 variants that were either terminated after the PSMGFR (see Table 1, SEQ ID NO: 37) or

one of the intracellular signaling pathways that is fairly well understood. To summarize, a mitogen binds to the extracellular portion of a transmembrane receptor and alters its conformation in such a manner that a signal is then transduced to the cell interior. As described above, a downstream step in this cascade is the phosphorylation of ERK2. It is known in the art that once ERK2 has been phosphorylated, cell proliferation proceeds. The inventors demonstrate that the addition of a bivalent antibody, which recognizes the MGFR, dimerizes the MUC1 receptor and in some way generates or reveals binding sites for signaling proteins that bind to the cytoplasmic tails of the MUC1 receptor. Figs. 15-17, respectively, show that in T47D, 1504, and 1500 breast tumor cells, dimerization of the MUC1 receptor via bivalent anti-PSMGFR results in ERK2 phosphorylation, see Ex. 12. The effect is dose-dependent and time-dependent. Further, synthetic compounds, which the inventors previously showed bind to the MGFR portion of the MUC1 receptor, compete with the bivalent anti-PSMGFR for binding to this region of the MUC1 receptor. In a competitive inhibition assay, the compounds effectively prevent (also in a dose-dependent way) the binding of the bivalent antibody to the MGFR, resulting in a loss of dimerization of the receptor and a loss of ERK2 phosphorylation, see Fig. 18 and Ex. 12.

Additionally, the monovalent form of the anti-PSMGFR competed with the bivalent antibody and effectively inhibited ERK2 phosphorylation. When excess monovalent anti-PSMGFR was added to breast tumor cells along with the amount of bivalent anti-PSMGFR that was shown to be sufficient to stimulate ERK2 phosphorylation, that phosphorylation was blocked, presumably because the monovalent antibody blocked the dimerization of the MUC1 receptor. Fig. 19 shows that monovalent anti-PSMGFR competes with the bivalent antibody and blocks the phosphorylation of ERK2 in cell line 1500.

Accordingly, in certain embodiments of the invention, the phosphorylation state of ERK2 can be monitored as a method for identifying therapeutics for MUC1 positive cancers.It was described above that monovalent compounds and monovalent anti-PSMGFR that bound to the MGFR competed with the bivalent antibody for binding to the site and in so doing inhibited the activation of the MAP kinase signaling pathway; ERK2 phosphorylation did not occur. This suggests a drug screen that will identify agents that affect signaling through the MUC1 receptor. In this drug screen, bivalent anti-PSMGFR is added to MUC1 positive tumor cells. Drug candidates are also added and the phosphorylation state of ERK2 is measured, as described previously. Cells in which ERK2 phosphorylation is inhibited indicates that that drug candidate successfully competed with

antibody that targets the PSMGFR, described above. Fig. 20 , the method for producing which is described in Ex. 5, is a western blot that shows that cell line 1504 expressed the highest levels of uncleaved MUC1, followed by T47D, then 1500 cells, with BT-474, K293, and HeLa cells showing no detectable amount of uncleaved MUC1. Fig. 14, the method for producing which is described in Ex. 5, is another western blot that shows that three breast tumor cell lines 1504, 1500 and T47D all expressed similar quantities of a proteolyzed MUC1 that ran with an apparent molecular weight of 20 – 30 kDa. It is noted that Wreschner et. al published data that showed that this MUC1 cleavage product is not expressed in normal, healthy breast tissue. BT-474 expressed a considerably lower level of cleaved MUC1. In addition, the protein bands of BT-474 are concentrated at 20 kDa with a low intensity presence at around 15 kDa (See Fig. 14). K293 cells showed no MUC1 expression as expected and HeLa cells showed minimal MUC1 between 20 – 30 kDa as previously reported in the literature. MDA-MB-453 also did not express detectable levels of cleaved or uncleaved MUC1(data not shown).

Cellular proteins often undergo post-translational modifications such as cleavage, glycosylation and phosphorylation. In particular, it is known that, under some circumstances, the extracellular domain of the MUC1 receptor is cleaved (at an unknown location) and shed into the bloodstream. The extracellular portion of the receptor can also be glycosylated, although it has been reported that in tumor cells, it is often under-glycosylated. The fact that the MUC1 receptor undergoes these indeterminate modifications makes it difficult to characterize the portion of the receptor that remains on the cell surface after cleavage in terms of length. Note that the degree of glycosylation alters the molecular weight of the receptor when analyzed by western blot, for example. Therefore, in order to compare expression levels of MUC1 among various cells types and to get a better determination of their true molecular weights, it is advantageous to deglycosylate protein samples prior to western gel analysis. Fig. 21, the method for producing which is described in Ex. 5, shows that after deglycosylation, the MUC1 protein bands converged to form a prominent band with an apparent molecular weight of about 20 kDa. These results suggest that all the breast tumor cell lines tested produced MUC1 cleavage products of approximately the same length but that had differential glycosylation. Note that non-deglycosylated 1504, 1500 and T47D samples showed three clear MUC1 protein bands. The PSMGFR sequence contains three Arginine residues that can be

glycosylated. Further analysis, refer to see Fig. 21 (lanes 3 versus 4), suggested that the MUC1 proteolysis product was in fact N- and not O-glycosylated.

To determine which amino acids were being glycosylated, the 1500 cell line was deglycosylated using enzymes that specifically remove O- or N-lined glycol units. Fig. 21 shows that the shift in molecular weight only occurs after treatment with the N-specific deglycosylase. This suggests that the PSMGFR region of the MUC1 receptor is only N-glycosylated.

In another set of embodiments, MUC1 isoform constructs of varying length were transfected into HEK cells and analyzed by western blot using anti-PSMGFR to determine cleavage patterns of the various MUC1 constructs. To investigate which portion of the extracellular domain of the MUC1 receptor is necessary and sufficient for or involved in the growth factor-like function described herein and to investigate the sequence of the portion of the receptor that remains on the cell surface after cleavage in tumor cells, HEK (human embryonic kidney) cells were transfected with plasmids designed to generate MUC1 receptor variants of different lengths. Fig. 22 is a cartoon that depicts the MUC1 constructs that were generated, see Exs. 6-7. In summary, constructs were generated to produce the entire MUC1 receptor (SEQ ID NO: 10 – Table 1), a MUC1 receptor variant with only 1.3 kilobases of the repeats section ("Rep isoform" - SEQ ID NO: 41 – Table 1), a MUC1 receptor variant that terminates after the IBR (interchain binding domain)("CM isoform" - SEQ ID NO: 38 – Table 1), a MUC1 receptor variant that terminates after the PSMGFR ("nat-PSMGFRTC isoform" – SEQ ID NO: 37 – Table 1), and the entire MUC1-Y alternative splice variant ("Y isoform" – SEQ ID NO: 40 – Table 1). Fig. 23 shows that apparently all of the MUC1 variants undergo cleavage, except the nat-PSMGFRTC isoform and the CM isoform constructs. There is evidence in the literature that sequences C-terminal to the end of the IBR are required for enzyme cleavage of the receptor.

It should be noted that it was observed that the cells transfected with the nat-PSMGFRTC isoform grew faster than the parent cell line and considerably faster (approximately 2-times) than the full length or repeats (Rep isoform) constructs. Further, the inventors have observed that HEK cells transfected with the nat-PSMGFRTC isoform are capable of anchorage-independent cell growth. Note that anchorage-independent cell growth is a phenomenon that is not yet understood but is a hallmark characteristic of true tumor cells. It was also observed that attempts to transiently transfect cells with the full length or repeats (Rep isoform) constructs were

difficult and frequently failed. In sharp contrast, the nat-PSMGFRTC isoform construct transfected easily each and every time it was tried. These results support the premise that it is the PSMGFR portion of the receptor that acts as a growth factor receptor.

To determine whether in HEK cells the MUC1 truncated receptors would behave as they do in tumor cells, a series of cell growth stimulation and inhibition were performed. In particular, bivalent antibodies and monovalent antibody binding fragments were added to these cells to determine their effect on cell growth and the phosphorylation state of ERK2. Fig. 24 shows that the addition of bivalent anti-PSMGFR (grey bars) to the nat-PSMGFRTC isoform construct transfected cells caused an 80% enhancement of cell proliferation, while the addition of monovalent anti-PSMGFR inhibited native cell growth by 50%. The figure also shows that the monovalent antibody competed with the bivalent antibody to diminish the extent of the enhanced cell growth. Fig. 25 shows that the addition of the bivalent antibody triggers ERK2 phosphorylation while Fig. 26 shows that the monovalent antibody competes with the bivalent to block ERK2 phosphorylation. nat-PSMGFRTC isoform transfected cell lines therefore constitute an excellent research tool for drug discovery for $MUC1^+$ cancers, since they behave similarly to tumor cells and this form of the receptor appears to be constitutively active.

To summarize, the extracellular domains that were represented in the transfected cells generated within the context of the present invention included: 1) the PSMGFR alone (nat-PSMGFRTC isoform – SEQ ID NO: 37 – Table 1); 2) the PSMGFR and the PSIBR (CM isoform – SEQ ID NO: 38 –Table 1); 3) the PSMGFR, PSIBR and a unique sequence that was ended just before the repeats region (UR isoform – SEQ ID NO: 39 – Table 1); 4) the PSMGFR, PSIBR, unique sequence and 1 kb of repeat sequences (Rep isoform – SEQ ID NO: 41 – Table 1); 5) the entire MUC1 extracellular domain (Full length MUC1 receptor – SEQ ID NO: 10 – Table 1); 6) and the entire extracellular domain of the Y isoform (SEQ ID NO: 40 – Table 1). Cells were grown and treated according to the standard protocols for analyzing the molecular weights of specific proteins by SDS-PAGE followed by western blot (see Example 5). The inventive antibody used in the western blot stage of the analysis specifically recognizes the PSMGFR sequence of the MUC1 receptor. The various transfectants were then analyzed by western blot.

As previously described, the construct expressed constitutively in MUC1+ cancer cell lines, in which the MUC1 receptor is believed to be terminated at or near the N-terminal end of the PSMGFR, produced a series of protein bands between 20 and 30 Kd when

glycosylated (See Fig. 21). After deglycosylation, the bands shifted to a molecular weight of about 20 Kd (Fig. 21). Significantly, the calculated molecular weight of the nat-PSMGFRTC isoform transfected construct is 19 Kd.

The MUC1 construct termed "Y isoform" produced a series of protein bands reacted with anti-PSMGFR in a western blot that moved through an SDS-PAGE gel with apparent molecular weights that ranged between 35 – 45 Kd (see Fig. 23). After deglycosylation, the motility of the proteins shifted to apparent molecular weights that ranged from 29 – 40 Kd. The calculated molecular weight of the transfected MUC1-Y isoform is 29 Kd. A faint protein band at 20 Kd appeared, which is consistent with the idea that some minimal cleavage of the Y isoform occurs to yield a proteolyzed fragment whose molecular weight is consistent with that of the nat-PSMGFRTC isoform. Comparison of the glycosylated protein lanes in Fig. 23 shows a clear difference between breast cancer patient-derived MUC1 proteins (1500 cell line) and the Y isoform transfected into HEK cells ("Y" lane). Referring still to the Figure 23, the lanes that were loaded with breast tumor cell samples do not show protein bands between 35 and 45 Kd; however, the glycosylated Y isoform protein bands are quite visible and intense between 35 and 45 Kd. These results may not rule out the possibility that patient-derived breast tumor cells may produce an alternative splice isoform, such as the Y isoform, in addition to MUC1, since it may be at low concentration and not visible by western blot analysis. However, these results clearly indicate that the dominant MUC1 species being produced by these breast tumor cell lines is not the Y isoform.

The CM isoform construct produced a doublet that ran at about 28 Kd. After deglycosylation, the bands shifted to a lower molecular weight of about 25 Kd. Comparison indicates that this construct is resistant to cleavage as the lower 20 Kd band apparent in the nat-PSMGFRTC isoform construct is not present.

Similar to the CM isoform construct, the UR isoform construct produced MUC1 protein bands that ran at 29 – 30 Kd. After deglycosylation, the bands shifted to molecular weights of about 24 Kd. A faint band at 20 Kd is visible, indicating that some cleavage of this construct takes place.

The "Full Length" construct and the Rep isoform construct appear to behave identically, producing PSMGFR specific bands at 25 – 30 Kd that shift to about 23 Kd after deglycosylation. By western blot it is impossible to distinguish this species from those produced by the patient derived breast tumor cells.

The (calculated) molecular weight of a portion of the MUC1 receptor that includes the cytoplasmic tail, the transmembrane domain and the PSMGFR (unglycosylated) is about 19Kd, (i.e. nat-PSMGFRTC isoform - see SEQ ID NO: 37 – Table 1) Also discovered within the context of the invention is that breast tumor cells express a shorter form of the MUC1 receptor that normal breast cells do not express and it runs on an SDS-PAGE gel at about 20Kd (deglycosylated), suggesting that the extracellular domain of this tumor specific form consists essentially of the PSMGFR.

To ascertain whether breast tumor cells, derived from actual breast cancer patients, produce a MUC1 cleavage product that is similar to the transfection constructs described above, we analyzed the MUC1 proteins from several breast tumor cell lines using the same western blot method described above and in Example 5. Breast tumor cell lines 1500, 1504, T47D, BT-474 and MDA-MB-453 were tested. The molecular weights of the MUC1 receptor fragments were then determined by performing standard western blot analysis, again using the antibody raised against the PSMGFR, as described above. Western blots showed that the breast tumor cell lines produced several MUC1 protein bands that run between 25 and 30 Kd. As with the nat-PSMGFRTC isoform construct, deglycosylation of the breast tumor derived samples caused the series of MUC1 protein bands (25 – 30 Kd) to shift to a band having an approximate molecular weight of 20 Kd, see Fig. 27. These western blots show that the lower molecular weight MUC1 species that the breast tumor cells produce are similar to the MUC1 construct discussed above in which the receptor is truncated after the PSMGFR. These results indicate that the portion of the MUC1 receptor that remains attached to the cell surface after cleavage consists primarily of the PSMGFR sequence. It is noted that it appears that the 1500 and T47D cells may produce two cleavage products, running as a doublet on the gel having a band at about 19-20 Kd and another at about 22 Kda. The 22 Kda band appears to correspond with the band produced by the CM isoform, which includes the PSIBR at its N-terminus. Significantly, (1) no band at 19-20 Kda is evident for the CM isoform construct, and (2) the 22 Kd band was also evident in the MUC1 cleavage products produced by transfected cells expressing "healthy" forms (i.e. Full Length receptor and Rep isoform – See Fig. 28). These results support the contention that the 22 Kd band product represents a product of normal, non-aberrant cleavage and that aberrant cleavage (i.e. producing the 19-20 Kd band may be prevented by the presence of the IBR. Since the resolution of molecular weights by SDS-PAGE analysis is only accurate to within about 1 Kd, which is about 9 amino acids, the actual portion of the

counted using a hemacytometer (3 counts per well) at 24 hours and again at 48 hours. Results, see Fig. 2, show that in a concentration-dependent manner, addition of antibody caused enhanced cell proliferation compared to the proliferation of the same cells treated with a control antibody. Figure 2 is a graph in which measured cell growth at 24 and 48 hours is plotted as a function of anti-MGFR concentration. At the optimal antibody concentration, when presumably one antibody binds bivalently to two MGFR portions of the MUC1 receptor, i.e. dimerizes the receptor, cell proliferation is at a maximum.

In a similar experiment, a concentration of the anti-MGFR antibody, identified to maximize cell proliferation, was added to a first group of T47D tumor cells, grown as described above. The same amount of the anti-MGFR antibody was added to a set of control cells, K293 cells. Figure 3 shows that the addition of the anti-MGFR antibody to MUC1 tumor cells (T47D) enhanced proliferation by 180% 24 hours, but had no effect on the control cells. The growth of the T47D cells plateaued to saturation, for cells with added antibody, at 48 hours. Control cells never reached saturation within the time frame of the experiment and were at 70% confluency at 48 hours.

Example 2: Identification of Ligands that bind to the MGFR portion of the MUC1 receptor

In an effort to identify ligands to the MUC1 receptor, synthetic, His-var-PSMGFR peptides, GTINVHDVETQFNQYKTEAASPYNLTISDVSVSDVPFPFSAQSGAHHHHHH (SEQ ID NO: 2), which is representative of the portion of the MUC1 receptor, that remains attached to the cell surface after cleavage of the interchain binding region, were loaded onto NTA-Ni beads (cat. #1000630; available from Qiagen GmbH, Germany) and incubated with cell lysates in the presence (Fig. 4) or absence (Fig. 5) of the protease inhibitor PMSF (phenyl methyl sulfonyl fluoride). Lysates from T47D cells were used because this breast tumor cell line is known to overexpress MUC1 and MUC1 ligand(s). T47D cells were cultured then sonicated for 1 minute to lyse the cells. Lysates were mixed with the PSMGFR peptide-presenting beads and incubated on ice with intermittent mixing for 1hr. As a negative control, an irrelevant peptide, HHHHHHRGEFTGTYITAVT (SEQ ID NO: 13), was attached to NTA-Ni beads and treated identically. Both sets of beads were washed 2X with phosphate buffer pH 7.4. Bound protein species were eluted by 3 additions of 100uL of phosphate buffer that also contained 250mM imidazole. For both the peptides, a

portion of the first elution was removed and reserved to run as a separate sample, while the remainder was combined with the other 2 elutions and concentrated by TCA (tri-chloro acetic acid)-precipitation (Chen, L. et al., Anal. Biochem. Vol 269; pgs 179-188; 1999). Eluates were run on a 12% SDS gel, see Figure 4. The gel was then silver stained (Schevchenko, A et al; Anal. Chem., Vol. 68; pg 850-858; 1996). Lanes were loaded as follows: (from left to right) (1) Benchmark pre-stained protein ladder (Gibco); (2) first eluate from the MUC1 peptide; (3) $1/10^{th}$ of TCA-concentrated sample; (4) blank; (5) $9/10^{th}$ TCA- concentrated sample; (6) first eluate negative control peptide; (7) $1/10^{th}$ of TCA-concentrated sample from the negative control peptide; (8) 0.5 picomoles BSA (as a standard); (9) $9/10^{th}$ TCA- concentrated sample from the negative control peptide; (10) silver stain SDS page standard (BioRad cat. #1610314). Referring now to Fig. 4, comparing lanes 2 and 6 (control), it can be seen that the MUC1 PSMGFR peptide bound distinguishably to three peptides: a first unique peptide that runs at an apparent molecular weight of 17kD; and a second peptide (more intense band) that runs at an apparent molecular weight of 23kD. Note that in lane 5, where the sample is the most concentrated, a third unique band is seen at about 35kD.

Figure 5 shows the results of an experiment, which was identical to that shown in Fig. 4, with the exception that the protease inhibitor PMSF was not added. PMSF binds to and blocks the action of several enzymes, such as proteases. This experiment was performed, in the absence of PMSF, to determine whether an enzyme present in the lysate was a ligand of the MUC1 receptor. Referring now to Fig. 5, comparing lanes 3 (control) and 7, it can be seen that the MUC1, PSMGFR peptide bound distinguishably to one peptide, with an apparent molecular weight of 35kD. Note that this band was visible in Fig. 4 (with PMSF), but was much fainter and only co-eluted from the most concentrated sample. These results are consistent with the idea that the PFMGFR portion of the MUC1 receptor is a substrate for a ligand of apparent molecular weight of about 35kD and which may bean enzyme. As mentioned elsewhere herein, drug screens based on inhibition of binding between the PSMGFR and this ligand or the ligand in a crude cell lysate can identify compounds that inhibit the action of this enzyme.

Table 3. Cell lines were purchased from the ATCC (American Type Culture Collection, Manasses, VA) and are all breast carcinoma cell lines. Some lines have been shown to

In this example, it is demonstrated that a ligand produced by MUC1 cancer cells that triggers cell proliferation in these cells is a multimer.

Protein bands at 17 kD, 23 kD, and 35 kD were excised from the gels described above in Example 2 of and submitted for peptide analysis. These gel bands purportedly contained ligands to the MGFR region of the MUC1 receptor. Recall that the 17 kD and 23 kD species bound to the MGFR peptide in the presence of the protease inhibitor, PMSF, while the 35 kD species bound when PMSF was not added to the cell lysate mixture.

The following peptide analysis was performed. Samples derived from the gel slices were proteolytically digested. Fragments were then separated by microcapillary HPLC which was directly coupled to a nano-electrospray ionization source of an ion trap mass spectrometer. MS/MS spectra was obtained on-line. These fragmentation spectra were then correlated to known sequences using the SEQUEST® algorithm in conjunction with other algorithms. Results were then manually reviewed to confirm consensus with sequences of known proteins.

Peptide sequences contained within both the 17 kD and the 23 kD bands (PMSF added to lysate) corresponded to a protein known as Metastasis Inhibition Factor NM23, which has been implicated in both the promotion and inhibition of metastasis of human cancers. Whether the role of NM23 is a tumor supressor or promoter may depend on the type of cancer. In ovarian, colon and neuroblastoma tumors, NM23 overexpression has been linked to a more malignant phenotype (Schneider J, Romero H, Ruiz R, Centeno MM, Rodriguez-Escudero FJ, "NM23 expression in advanced and borderline ovarian carcinoma", *Anticancer Res*, 1996; 16(3A): 1197-202). However, breast cancer studies indicate that reduced expression of NM23 correlates with poor prognosis (Mao H, Liu H, Fu X, Fang Z, Abrams J, Worsham MJ, "Loss of nm23 expression predicts distal metastases and poorer survival for breast cancer", Int J Oncol 2001 Mar;18(3):587-91).

The sequences that were identified from the protein gel band described in Figures 4 and 5 and that are derived from a protein implicated in many cancers called Metastasis Inhibition Factor NM23 are shown below in Table 5. NM23 exists as a hexamer and may recognize an unmodified form of the MGFR portion of the MUC1 receptor.

Peptide sequences that were identified from the 35 kD gel band (PMSF NOT added to lysate) corresponded to more than one protein species, including 14-3-3, which is a signaling protein implicated in many cancers, and cathepsin D, which is a protease and is also implicated in tumor progression. 14-3-3 exists as a dimer and can simultaneously bind

apparatus as described by manufacturer. Electrophoretic transfer was performed at 25V for 45 minutes.

Western Blotting:

Membranes were removed and placed immediately in 25 ml Blotto (PBS, 0.05% Tween-20, 5% Milk) and incubated with gentle agitation for 2 hours. Blotto was removed and replaced with 25 ml primary antibody solution [1:1000 dilution of an inventive α-PSMGFR antibody or 1:200 dilution of VU-4H5 antibody (Santa Cruz Biotechnologies; Santa Cruz, CA), in Blotto] and incubated overnight at 4°C. The solution was then discarded and the membrane washed 5 times for 10 minutes each in PBS-T (PBS, 0.05% Tween-20). Membranes were then incubated in secondary antibody solution [1:20000 dilution of HRP(horseradish peroxidase)-conjugated Goat-α-Rabbit IgG antibody or HRP-conjugated Rabbit-α-Mouse IgG antibody (Jackson Immunoresearch; West Grove, PA) in Blotto] for 1 hour at room temperature. Solution was discarded and the membrane was washed 5 times for 10 minutes each in PBS-T. The membrane was then placed in a 1:1 mixture of Immun-Star HRP Luminol/Enhancer and Peroxide Buffer from BioRad Laboratories (Hercules, CA) for 5 minutes. Substrate was removed and membrane placed in saran wrap and exposed to film and developed in Kodak X-OMAT.

Example 6 - Transfectants: The construction of six mammalian expression plasmids encoding six different lengths of the Muc1 receptor

pMuc1-Full, Encoding Full-length MUC1 Receptor

The pMuc1-Full construct contains the complete cDNA for MUC1 and encodes the full length Muc1 protein (Figure 22 and SEQ ID NO: 10). The pMuc1-Full plasmid was put together from two separate plasmids containing different parts of the MUC1 cDNA. The amino-terminus of MUC1 was acquired from EST0039670 obtained from the Genome Research Center and the Center for Functional Analysis of Human Genome (GRC/CFAHG) Korea Research Institute of Bioscience and Biotechnology (Taejeon, Korea). The EST0039670 plasmid contained a cDNA starting at the amino-terminus of the MUC1 open reading frame to about 800 base pairs into the tandem repeats segment of MUC1. The carboxy-terminus of the MUC1 cDNA was obtained from Integrated Molecular Analysis of Genomes and their Expression (IMAGE) clone number 2428103 obtained from American

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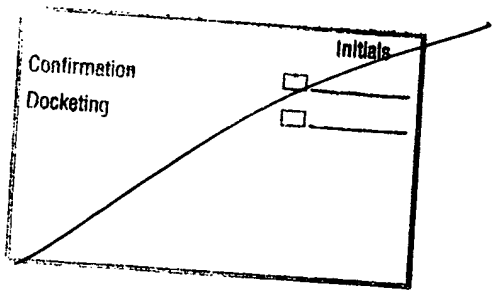

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Attn. Oyer, Timothy J.
600 Atlantic Avenue
Boston, Massachusetts 02210
UNITED STATES OF AMERICA

NOTIFICATION OF DECISION CONCERNING
REQUEST FOR RECTIFICATION

(PCT Rule 91.1(f))

Applicant's or agent's file reference M1015.70089WO	Date of mailing *(day/month/year)* 22/02/2005
International application N°. PCT/US2004/027954	**REPLY DUE** NONE However, see last paragraph below
Applicant MINERVA BIOTECHNOLOGIES CORPORATION	International filing date *(day/month/year)* 26/08/2004

The applicant is hereby notified that this International Searching Authority has considered the request for rectification of obvious errors in the international application/in other papers submitted by the applicant to this Authority, and that it has decided:

1. [X] to authorize the rectification:

   [X] as requested by the applicant.

   [ ] to the extent set forth below*:

2. [ ] to refuse to authorize the rectification or part of it for the following reasons*:

A copy of this notification, together with a copy of the applicant's request for rectification, has been sent to the receiving Office and to the International Bureau.

* **If the authorization of the rectification has been refused in whole or in part,** the applicant may request the International Bureau, before the technical preparations for international publication have been completed and subject to the payment of a fee, to publish the request for rectification together with the international application. See Rule 91.1(f), third and fourth sentences, and, for the amount of the fee, see the PCT Applicant's Guide, Volume I/A, Annex B2(IB).

Name and mailing address of the International Searching Authority European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Wolfgang-Peter Schießl

Form PCT/ISA/217 (July 1998)

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION CONCERNING
SUBMISSION OR TRANSMITTAL
OF PRIORITY DOCUMENT

(PCT Administrative Instructions, Section 411)

To:

OYER, Timothy, J.
Wolf, Greenfield & Sacks
600 Atlantic Avenue
Boston, MA 02210
ETATS-UNIS D'AMERIQUE

Date of mailing *(day/month/year)* 24 January 2005 (24.01.2005)	
Applicant's or agent's file reference M1015.70089 WO00	**IMPORTANT NOTIFICATION**
International application No. PCT/US04/027954	International filing date *(day/month/year)* 26 August 2004 (26.08.2004)
International publication date *(day/month/year)*	Priority date *(day/month/year)* 26 August 2003 (26.08.2003)
Applicant MINERVA BIOTECHNOLOGIES CORPORATION et al	

1. By means of this Form, which replaces any previously issued notification concerning submission or transmittal of priority documents, the applicant is hereby notified of the date of receipt by the International Bureau of the priority document(s) relating to all earlier application(s) whose priority is claimed. Unless otherwise indicated by the letters "NR", in the right-hand column or by an asterisk appearing next to a date of receipt, the priority document concerned was submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b).

2. *(If applicable)* The **letters "NR"** appearing in the right-hand column denote **a priority document which, on the date of mailing of this Form, had not yet been received by the International Bureau** under Rule 17.1(a) or (b). Where, under Rule 17.1(a), the priority document must be submitted by the applicant to the receiving Office or the International Bureau, but the applicant fails to submit the priority document within the applicable time limit under that Rule, **the attention of the applicant is directed** to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

3. *(If applicable)* **An asterisk (*)** appearing next to a date of receipt, in the right-hand column, denotes **a priority document submitted or transmitted to the International Bureau but not in compliance with Rule 17.1(a) or (b)** (the priority document was received after the time limit prescribed in Rule 17.1(a) or the request to prepare and transmit the priority document was submitted to the receiving Office after the applicable time limit under Rule 17.1(b)). Even though the priority document was not furnished in compliance with Rule 17.1(a) or (b), the International Bureau will nevertheless transmit a copy of the document to the designated Offices, for their consideration. In case such a copy is not accepted by the designated Office as the priority document, Rule 17.1(c) provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

Priority date	Priority application No.	Country or regional Office or PCT receiving Office	Date of receipt of priority document
26 August 2003 (26.08.2003)	60/498,260	US	09 December 2004 (09.12.2004)

Initials
Confirmation
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T10

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. +41 22 740 14 35

Authorized officer

sapin alexia

Facsimile No. +41 22 338 70 10
Telephone No. +41 22 338

Form PCT/IB/304 (January 2004)

PATENT COOPERATION TREATY

I+D

PCT

From the INTERNATIONAL BUREAU

To:

OYER, Timothy, J.
Wolf, Greenfield & Sacks
600 Atlantic Avenue
Boston, MA 02210
United States of America

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

Date of mailing (day/month/year)
12 January 2005 (12.01.2005)

Applicant's or agent's file reference
M1015.70089 WO

IMPORTANT NOTIFICATION

International application No.
PCT/US2004/027954

International filing date (day/month/year)
26 August 2004 (26.08.2004)

1. The following indications appeared on record concerning:

[X] the applicant [X] the inventor [ ] the agent [ ] the common representative

Name and Address
BAMDAD, Cynthia, C.
142 Church St.
Newton, MA 02458
United States of America

State of Nationality: US
State of Residence: US
Telephone No.:
Facsimile No.:
Teleprinter No.:

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[ ] the person [ ] the name [X] the address [ ] the nationality [ ] the residence

Name and Address
BAMDAD, Cynthia, C.
356 Marlborough Street, #2
Boston, MA 02116
United States of America

State of Nationality: US
State of Residence: US
Telephone No.:
Facsimile No.:
Teleprinter No.:

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[X] the International Searching Authority
[ ] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[ ] the elected Offices concerned
[ ] other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Facsimile No. (41-22) 338.70.10

Authorized officer
Jean-Marie MCADAMS (Fax 338 7010)
Telephone No. (41-22) 338 9913

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

COMMUNICATION IN CASES FOR WHICH NO OTHER FORM IS APPLICABLE

To:

OYER, Timothy, J.
Wolf, Greenfield & Sacks
600 Atlantic Avenue
Boston, MA 02210
ETATS-UNIS D'AMERIQUE

Date of mailing (*day/month/year*) 12 November 2004 (12.11.2004)	
Applicant's or agent's file reference M1015.70089 WO	**REPLY DUE** see paragraph 1 below
International application No. PCT/US2004/027954	International filing date (*day/month/year*) 26 August 2004 (26.08.2004)
Applicant MINERVA BIOTECHNOLOGIES CORPORATION	

1. ☐ REPLY DUE within _____ months/days from the above date of mailing

☐ NO REPLY DUE, however, see below

☒ IMPORTANT COMMUNICATION

☐ INFORMATION ONLY

2. COMMUNICATION:

The International Bureau acknowledges receipt of your request of change dated 04 November 2004 (04.11.04).

However, please note that a power of attorney from the inventor/applicant Bamdad, Cynthia, C. with the original address is required, before any change under rule 92bis may be made. Upon receipt said power of attorney, change will processed.

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer Monique BROSSE (Fax 338 7010)
Facsimile No. (41-22) 338.70.10	Telephone No. (41-22) 338.83.25

PATENT COOPERATION TREATY

IFD

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF RECEIPT OF RECORD COPY

(PCT Rule 24.2(a))

To:

OYER, Timothy, J.
Wolf, Greenfield & Sacks
600 Atlantic Avenue
Boston, MA 02210
United States of America

Date of mailing (day/month/year)	IMPORTANT NOTIFICATION
12 November 2004 (12.11.2004)	
Applicant's or agent's file reference	**International application No.**
M1015.70089 WOOO	PCT/US2004/027954

The applicant is hereby **notified** that the International Bureau has received the record copy of the international application as detailed below.

Name(s) of the applicant(s) and State(s) for which they are applicants:

MINERVA BIOTECHNOLOGIES CORPORATION (for all designated States except US)
BAMDAD, Cynthia, C. (for US)

International filing date	:	26 August 2004 (26.08.2004)
Priority date(s) claimed	:	26 August 2003 (26.08.2003)
Date of receipt of the record copy by the International Bureau	:	06 October 2004 (06.10.2004)
List of designated Offices	:	

AP :BW,GH,GM,KE,LS,MW,MZ,NA,SD,SL,SZ,TZ,UG,ZM,ZW
EA :AM,AZ,BY,KG,KZ,MD,RU,TJ,TM
EP :AT,BE,BG,CH,CY,CZ,DE,DK,EE,ES,FI,FR,GB,GR,HU,IE,IT,LU,MC,NL,PL,PT,RO,SE,SI,SK,TR
OA :BF,BJ,CF,CG,CI,CM,GA,GN,GQ,GW,ML,MR,NE,SN,TD,TG
National :AE,AG,AL,AM,AT,AU,AZ,BA,BB,BG,BR,BW,BY,BZ,CA,CH,CN,CO,CR,CU,CZ,DE,DK,DM,DZ,EC,EE,EG,ES,FI,GB,GD,GE,GH,GM,HR,HU,ID,IL,IN,IS,JP,KE,KG,KP,KR,KZ,LC,LK,LR,LS,LT,LU,LV,MA,MD,MG,MK,MN,MW,MX,MZ,NA,NI,NO,NZ,OM,PG,PH,PL,PT,RO,RU,SC,SD,SE,SG,SK,SL,SY,TJ,TM,TN,TR,TT,TZ,UA,UG,US,UZ,VC,VN,YU,ZA,ZM,ZW

DOCKETED
DEC 6 2004

Initials
Confirmation ☐
Docketing ☑
4/26/05 a/e

FHO

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer: Monique BROSSE (Fax 338 7010)
Facsimile No. (41-22) 338.70.10	Telephone No. (41-22) 338 8325

Continuation of Form PCT/IB/301

NOTIFICATION OF RECEIPT OF RECORD COPY

Date of mailing (day/month/year)	IMPORTANT NOTIFICATION
12 November 2004 (12.11.2004)	
Applicant's or agent's file reference	**International application No.**
M1015.70089	PCT/US2004/027954

ATTENTION

The applicant should carefully check the data appearing in this Notification. In case of any discrepancy between these data and the indications in the international application, the applicant should immediately inform the International Bureau.

In addition, the applicant's attention is drawn to the information contained in the Annex, relating to:

[X] time limits for entry into the national phase - **see updated important information (as of April 2002)**

[X] requirements regarding priority documents (if applicable)

A copy of this Notification is being sent to the receiving Office and to the International Searching Authority.

INFORMATION ON TIME LIMITS FOR ENTERING THE NATIONAL PHASE

The applicant is reminded that the **"national phase" must be entered** before each of the designated Offices indicated on the cover sheet of this Notification by paying national fees and furnishing translations, as prescribed by Articles 22 and 39 and the applicable national laws. In addition, the applicant may also have to comply **with other special requirements** applicable in certain Offices. It is the **applicant's responsibility** to ensure the necessary steps to enter the national phase are taken in a timely fashion. Most Offices do not issue reminders to applicants in connection with the entry into the national phase.

The **applicable time limit** for entering the national phase will, **subject to what is said in the following paragraph,** be **30 MONTHS** from the priority date, not only in respect of any elected Office if a demand for international preliminary examination is filed before the expiration of 19 months from the priority date (see Article 39(1)), but also in respect of any designated Office, in the absence of filing of such demand, where Article22(1) as modified with effect from 1 April 2002 applies in respect of that designated Office. For further details, see PCT Gazette No. 44/2001 of 1 November 2001, pages 19926, 19932 and 19934, as well as the PCT Newsletter, October and November 2001 and February 2002 issues.

In practice, **time limits other than the 30-month time limit will continue to apply, for various periods of time,** in respect of certain designated or elected Offices. For **regular updates on the applicable time limits (20, 21, 30 or 31 months, or other time limit),** Office by Office, refer to the PCT Gazette("Section IV" part published on a weekly basis), to the PCT Newsletter (on a monthly basis) and to the relevant National Chapters in Volume II of the PCT Applicant's Guide (the paper version of which is updated usually twice a year and the Internet version of which is updated usually on a weekly basis). Finally, a cumulative table of all applicable time limits for entering the national phase is available from WIPO's Internet site, via links from various pages the site including those of the Gazette, Newsletter and Guide, at http://www.wipo.int/pct/en/index.html.

Information about the requirements for **filing a demand for international preliminary examination** is set out in the PCT Applicant's Guide, Volume I/A, Chapter IX. Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination (at present, all PCT Contracting States are bound by Chapter II).

REQUIREMENTS REGARDING PRIORITY DOCUMENTS

For applicants who have not yet complied with the requirements regarding priority documents, the following is recalled.

Where the priority of an earlier national, regional or international application is claimed, the applicant must submit a copy of the said earlier application, certified by the authority with which it was filed ("the priority document") to the receiving Office (which will transmit it to the International Bureau) or directly to the International Bureau, before the expiration of 16 months from the priority date, provided that any such priority document may still be submitted to the International Bureau before that date of international publication of the international application, in which case that document will be considered to have been received by the International Bureau on the last day of the 16-month time limit (Rule 17.1(a)).

Where the priority document is issued by the receiving Office, the applicant may, instead of submitting the priority document, request the receiving Office to prepare and transmit the priority document to the International Bureau. Such request must be made before the expiration of the 16-month time limit and may be subjected by the receiving Office to the payment of a fee (Rule 17.1(b)).

If the priority document concerned is not submitted to the International Bureau or if the request to the receiving Office to prepare and transmit the priority document has not been made (and the corresponding fee, if any, paid) within the applicable time limit indicated under the preceding paragraphs, any designated State may disregard the priority claim, provided that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within the time limit which is reasonable under the circumstances (Rule 17.1(c)).

Where several priorities are claimed, the priority date to be considered for the purposes of computing the 16-month time limit (and all other PCT time limits) is the filing date of the earliest application whose priority is claimed (Article 2(xi)(b)).

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

To:

OYER, Timothy, J.
Wolf, Greenfield & Sacks
600 Atlantic Avenue
Boston, MA 02210
United States of America

Date of mailing (day/month/year)
12 November 2004 (12.11.2004)

Applicant's or agent's file reference
M1015.70089 WO

IMPORTANT NOTIFICATION

International application No.
PCT/US2004/027954

International filing date (day/month/year)
26 August 2004 (26.08.2004)

1. The following indications appeared on record concerning:

[X] the applicant [ ] the inventor [ ] the agent [ ] the common representative

Name and Address

MINERVA BIOTECHNOLOGIES CORPORATION
142 Church St.
Newton, MA 02458
United States of America

State of Nationality: US
State of Residence: US
Telephone No.:
Facsimile No.:
Teleprinter No.:

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[ ] the person [ ] the name [X] the address [ ] the nationality [ ] the residence

Name and Address

MINERVA BIOTECHNOLOGIES CORPORATION
6th Floor Rosenstiel Bldg.
M/S 029
415 South Street
Waltham, MA 02454-9110
United States of America

State of Nationality: US
State of Residence: US
Telephone No.:
Facsimile No.:
Teleprinter No.:

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[ ] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[ ] the elected Offices concerned
[ ] other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 338.70.10

Authorized officer

Monique BROSSE (Fax 338 7010)

Telephone No. (41-22) 338 8325

Form PCT/IB/306 (March 1994)

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

PCT

NOTIFICATION OF RECEIPT OF SEARCH COPY

(PCT Rule 25.1)

DOCKETED
NOV 3 2004

To:
WOLF GREENFIELD & SACKS, P.C.
Attn. Oyer, Timothy J.
600 Atlantic Avenue
Boston, Massachusetts 02210
UNITED STATES OF AMERICA

Initials
Confirmation
Docketing
12-26-04 ale
2-6-05

Date of mailing (day/month/year) 22/10/2004

Applicant's or agent's file reference
M1015.70089 WO00

IMPORTANT NOTIFICATION

International application No.	International filing date (day/month/year)	Priority date (day/month/year)
PCT/US2004/027954	26/08/2004	26/08/2003

Applicant

MINERVA BIOTECHNOLOGIES CORPORATION

1. **Where the International Searching Authority and the receiving Office are not the same office:**

The applicant is hereby notified that the search copy of the international application was received by this International Searching Authority on the date indicated below.

Where the International Searching Authority and the receiving Office are the same office:

The applicant is hereby notified that the search copy of the international application was received on the date indicated below.

06/10/2004 ✓ *(date of receipt).*

2. ☐ The search copy was accompanied by a nucleotide and/or amino acid sequence listing or tables related thereto in computer readable form.

3. **Time limit for establishment of international search report and written opinion of the International Searching Authority**

The applicant is informed that the time limit for establishing the international search report and the written opinion of the International Searching Authority is three months from the date ofreceipt indicated above or nine months from the priority date, whichever time limit expires later (Rules 42.1 and 43*bis*.1(a))

4. A copy of this notification has been sent to the International Bureau and, where the first sentence of paragraph 1 applies, to the receiving Office.

Name and mailing address of the International Searching Authority	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	ISA/EP

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